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EFFECTS OF IMMOBILIZATION ON ARTICULAR CARTILAGE:
AUTOHISTORADIOGRAPHIC FINDINGS WITH S³⁵

by C. Di Giovanni and E. DeSantis

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EFFECTS OF IMMOBILIZATION ON ARTICULAR CARTILAGE:
AUTOHISTORADIOGRAPHIC FINDINGS WITH S³⁵

C. Di Giovanni and E. DeSantis
Clinical Orthopedics Institute, The Catholic University, Rome

Introduction

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Prolonged immobilization of the articular segments, which often occurs in clinical practice, leads in the long run to more or less pronounced limitation of articular excursion. This limitation is directly proportional to the duration of the period of immobilization (taking individual variations into account).

Protracted absence of movement affects both the muscle-tendon region and the articular region with its constituent elements.

The clinical aspects of the phenomenon and the effect of immobilization on the muscular component are remarkable. However, findings are insufficient with regard to damage to intra-articular formations and especially to epiphyseal cartilage. Experimental work in this area by Menzel (1871), Ely and Mensor (1933) and Scaglietti and Casuccio (1936) has been confirmed by research by Evans, Eggers and Butler (1960); Hall (1964); Thaxter, Mann and Anderson (1965); Thompson and Basset (1970); Troyer (1975) and Finsterbush and Friedman (1975).

Movement, as well as weight, represents an extremely important element in the development of functional activity of the articular cartilage.

In fact, the stimulus provided by movement and weight conditions the development and, along with it, the "functional differentiation" of the cartilaginous tissue. By virtue of the structural arrangement, as determined by the above-mentioned stimulus, the cartilage becomes an ideal organ for mechanical functions.

*Numbers in the margin indicate pagination in the Italian text.

The entire period of somatic growth is characterized by progressive functional differentiation which occurs simultaneously with the development of the organism.

These processes of structural arrangement, begun at birth, are completed with the conclusion of growth, and affect the cells as well as the basic substance and the fibrillar trama.

Thanks to these processes, the articular cartilage becomes highly differentiated, in order to perform any significant mechanico-functional tasks which may be necessary.

With the conclusion of growth, the weight borne (which acts on a structurally stabilized tissue) is still in a position to affect the differentiation of the constituent elements of the tissue. In fact, a decrease in or loss of this weight causes cartilaginous atrophy. This atrophy is characterized by a decrease in thickness and by a structural /24 disturbance whereby the cartilaginous tissue acquires an overall configuration similar to that of embryonic articular cartilage. The layered arrangement is completely upset and layers become indistinguishable. The cells, which are extremely monomorphic, are rearranged in a non-preferential orientation.

Prevention of movement also significantly disturbs the permeability mechanism of articular cartilage. Immobilization affects the metabolic exchanges between the cartilaginous cells and the synovial fluid, which constitute the principal means of nutrition for the articular cartilage.

Therefore, the purpose of our experimental study is to ascertain the nature and extent of the changes which occur in articular cartilage which has been subjected to immobilization.

Materials and Methods

Experimentation was carried out on New Zealand rabbits, for which we sought to maintain weight and age parameters.

For this study, it was preferable to immobilize the knee joint by strictly practical means, due to the great similarity of this procedure to that followed with human beings.

Two different methods were used to achieve the experimental conditions of articular immobilization with weight allowed:

- A) Immobilization of the flexed joint by means of a pelvi-pedal plaster device or plaster knee cast, so as to reproduce the conditions usually found in human pathology.
- B) Immobilization of the joint with Kirschner wire crossing the femoral condyle and the proximal metaphysis of the homolateral tibia, fixed medially and laterally between them with aluminum plates which are drilled with holes so as to enable stabilization of the joint in various degrees of angularity. This method was implemented by anesthetizing the animal with a single intraperitoneal injection of urethan (ethyl urethane).

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The second method of immobilization was discarded after several initial attempts, due to technical difficulties and to the extreme variability of possible results with respect to compression which, moreover, does not reproduce physiological conditions in an experimental environment.

The knee of the contralateral limb, which was not immobilized, was initially utilized as a control. However, the functional overload of this joint subsequently made it necessary to utilize as a control the joints of rabbits of the same weight and age as the treated animals, and which were kept in the same cages with the treated animals.

With respect to the period of immobilization, the animals were separated into four groups of four rabbits each. Four rabbits were utilized as controls.

- Group I: immobilization up to 30 days
- Group II: immobilization up to 60 days
- Group III: immobilization up to 90 days
- Group IV: immobilization up to 120 days

The joints excised following stipulated periods of immobilization were subjected to standard histological and histochemical procedures, including fixation of the material in 10% neutral formaldehyde, decalcification in 5% nitric acid, dehydration, inclusion and staining of the sections. /26

Autohistoradiography with the radioactive S^{35} isotope was carried out in order to evaluate the turnover in proteoglycans (mucoproteins) in the basic substance.

In fact, sulfur enters into the constitution of the most important glycosaminoglycan acids of the articular cartilage: chondroitin sulfate A, C and keratosulfate. The acid valences of these compounds are represented by the $-COOH$ and $-SO_3H$ groups.

S^{35} uptake occurs where biosynthesis of these macromolecular chains takes place, and consequently provides an index of the metabolic activity of the cellular elements.

In order to obtain autohistoradiographic data on the joint, a solution of radioactive sodium sulfate ($Na_2S^{35}O_4$) was injected intraperitoneally in a dose of one millicurie per kg of body weight.

The treated rabbits and the control rabbits were sacrificed 24, 48 and 72 hours after administration of the isotope. Histological sections were obtained in accordance with standard procedures. These sections were subjected to autoradiography with Kodak NTB2 emulsion which had been exposed for an average of 25 days. Following development and fixation, the prepared sections were stained with eosin hematoxylin. /27

Results

Group I (immobilization up to 30 days):

Immobilization for brief periods, e.g., less than ten days, is not sufficient to produce noticeable or documentable morphological changes.

After two weeks, a small quantity of synovial fluid was observed at the opening of the articular cavity, as compared to the control. Aside from greater adhesiveness, the synovial membrane showed no remarkable changes.

The articular surface appeared uniform, smooth, well-moistened and slightly thinner (Figure 1 and Figure 5).

There was a roundish depression on the anterior face of the medial femoral condyle (Figure 2). The epiphyseal and subchondral bones appeared to be slightly porous. The layered arrangement of the articular cartilage was well maintained (Figure 3). The basic substance was slightly basophilic with respect to the control, especially in the superficial layer and in the pericellular area. While cellular elements were well represented numerically, they began to show the initial signs of atrophy (Figure 3).

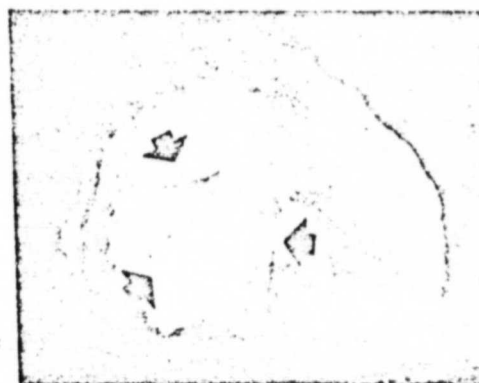
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The layer of calcified cartilage was sometimes interrupted by penetrations of numerous vascular phenomena (Figures 4,6,7 and 8), These phenomena were more noticeable in the marginal zones of the joint which were not subjected to constant contact with the corresponding articular face.

These vessels, whose walls are rather thin, show an almost constant dilation. The majority of them can be localized in the basal layer of the articular cartilage where complete reabsorption of the basic substance occurs.



Fig. 1. Group I (joint subjected to immobilization for 20 days).
A,B: The articular surface of the treated joint (*) appears uniform, smooth and well-moistened, with characteristics comparable to those of the control.



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Fig. 2. There is an ovoidal depression (indicated by arrows) on the anterior face of the medial condyle.

Group II (immobilization from 30 to 60 days):

A small quantity of fluid was found at the articular opening. However, the synovial membrane was very evident and difficult to remove from the most marginal portions of the articular head, where

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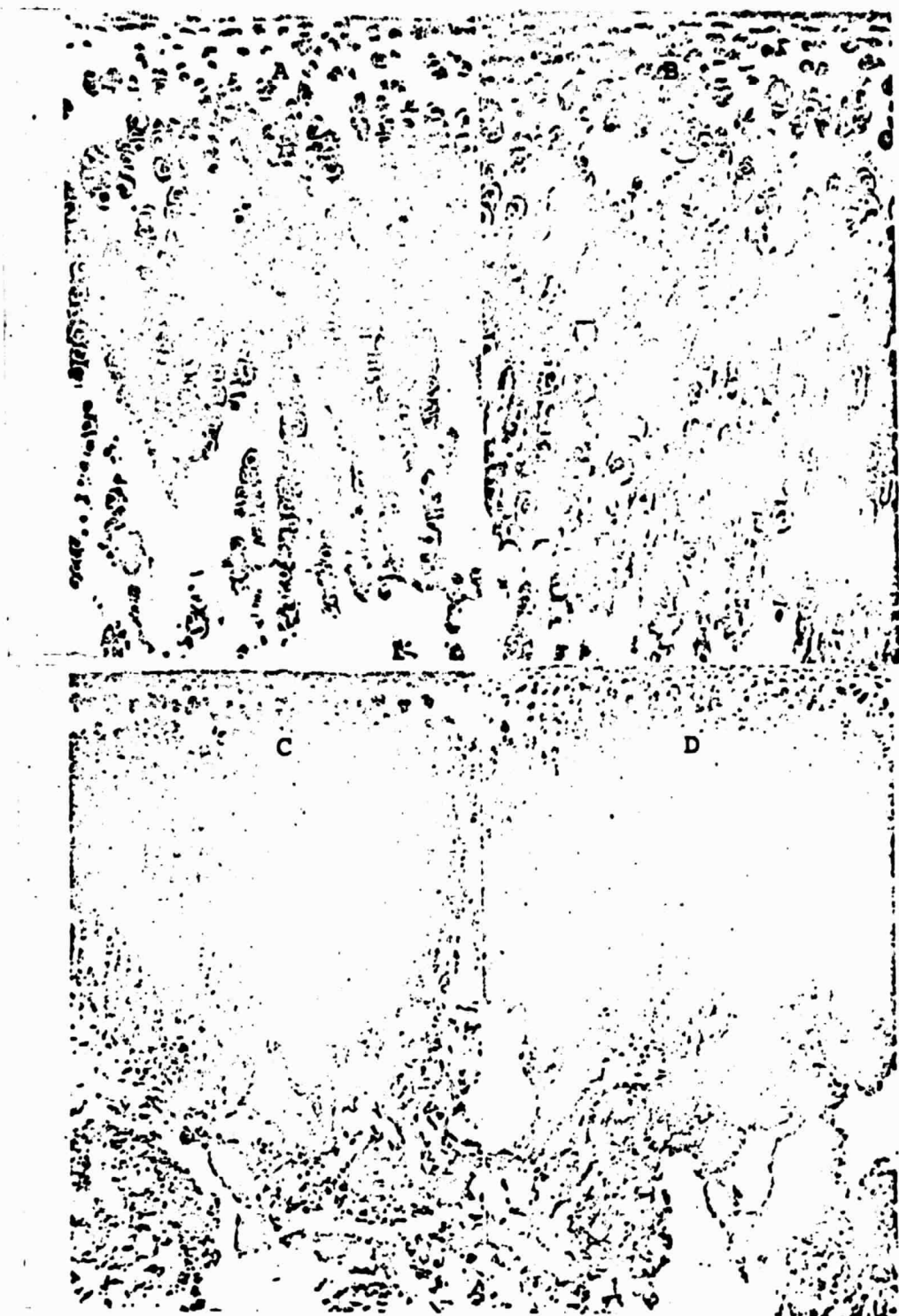


Fig. 3. Group I (immobilization for 20 days). The treated articular cartilage (B,D) is of the same thickness as the control (A,C). Diffuse hypochromia of the basic substance is noticeable, with prevalent effects on the superficial layer and on the basal layer.

there was no adherence to the overlying surface of the articular heads. A higher consistency and adhesive capacity of the membrane corresponds to lower laxity of the stromal axis.



Fig. 4. Group I (immobilization for 20 days). Some vascular formations project from the subchondral bone into the layer of calcified cartilage.

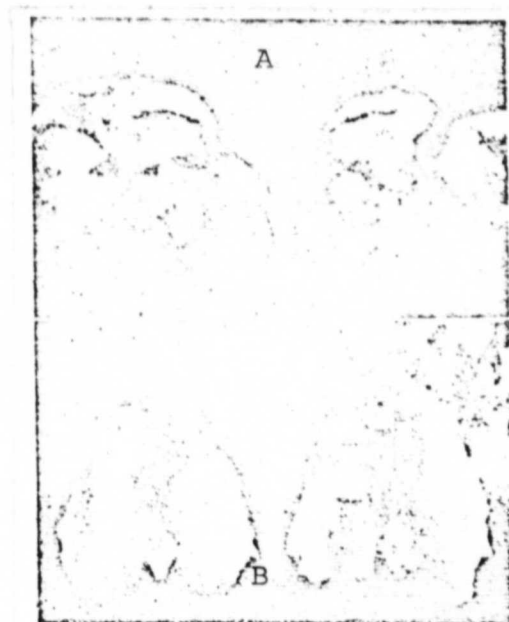


Fig. 5. Group I (immobilization for 28 days). A,B: The cartilage of the tibial plate of the treated joint (*) is uniformly thinner.

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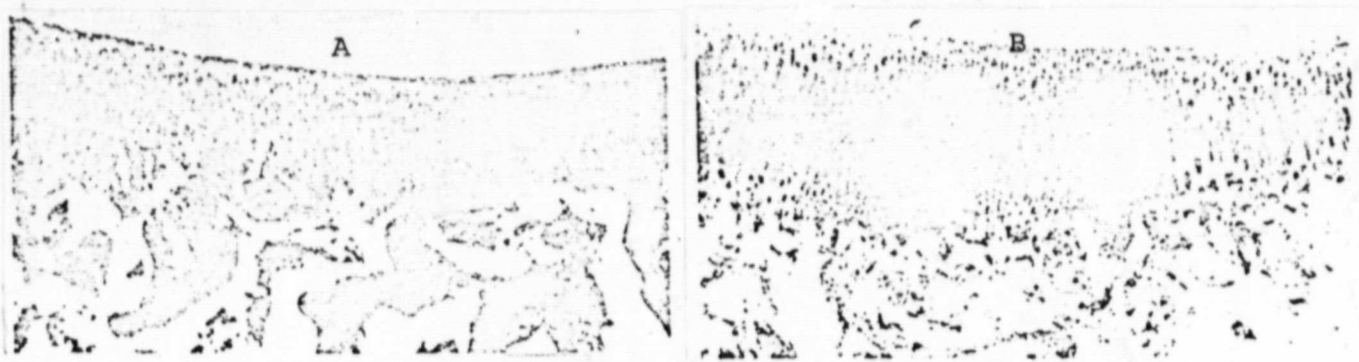


Fig. 6. Group I (immobilization for 28 days). Comparison of the control cartilage (B) and the treated cartilage (A) reveals reduced thickness and hypochromia in the latter.

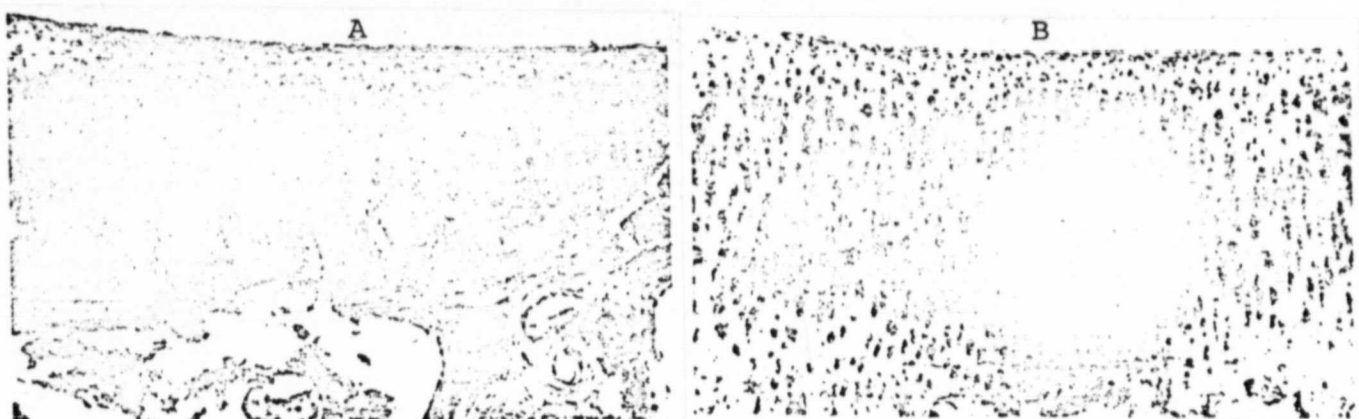


Fig. 7. Group I (immobilization for 28 days). A: The marginal zone shows numerous vascular formations which have penetrated the layer of calcified cartilage. B: For comparison, a corresponding area of the control.

The connective tissue and the adipose tissue which normally constitute the latter portion are replaced by a dense connection which is sometimes infiltrated by very small cellular elements which often contain neoformative vessels (Figure 15 and Figure 17). The articular surface maintains its profile but appears less clear and more grainy, without continuous solutions. The overall thickness of the



Fig. 8. Group I (immobilization for 28 days). Detail of vascular penetration. The vessels determine the complete reabsorption of localized areas of the basal layer.

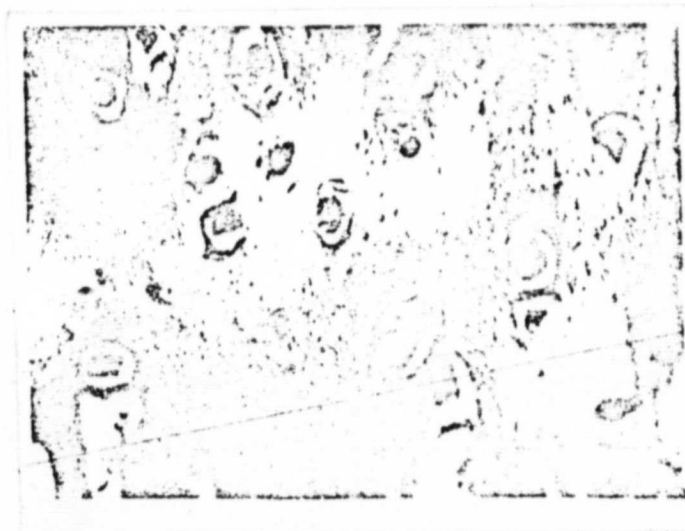


Fig. 9. Group I (immobilization for 28 days). In the deep zone of the cartilage, the reduction of the basic substance determines the exposure of collagen fibrils.

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cartilage is reduced, to such an extent that in some areas, the coloration of the subchondral bone shows through (Figure 10).

The basic substance is less compact, more stain-resistant and absent in some basal areas (Figure 11 and Figure 13). Interstitial edema is sometimes accompanied by exposure of the collagen fibrils (Figure 12 and Figure 14).

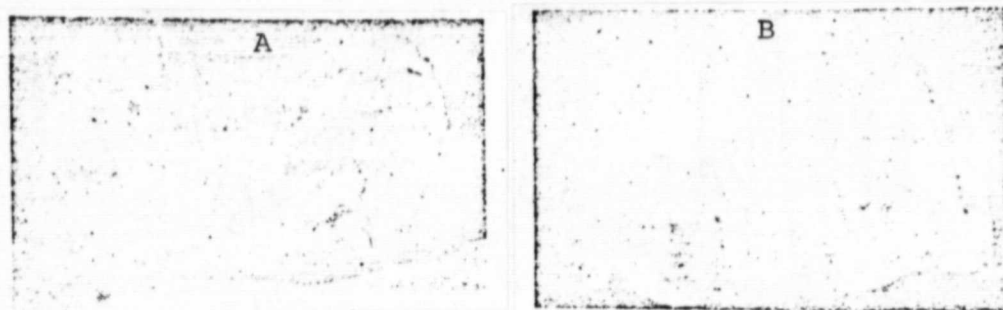


Fig. 10. Group II (immobilization for 35 days). A: The treated medial condyle (*) shows a depression in the articular surface (indicated by arrows). B: The articular surface of the kneecap is diffusely thinner, as compared to the control.

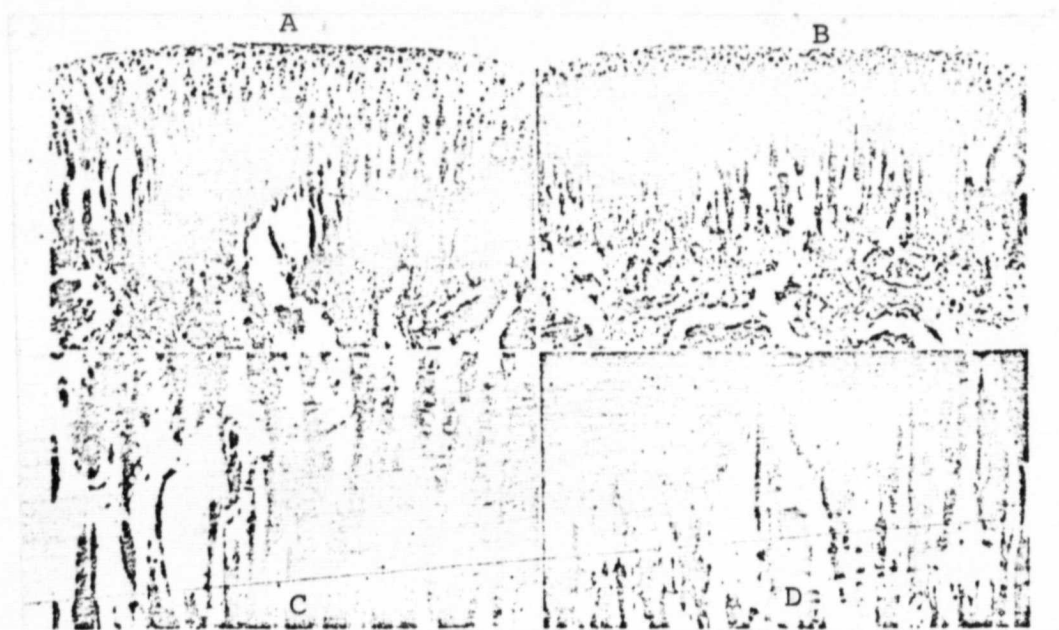


Fig. 11. Group II (immobilization for 35 days). The depletion of the basic substance causes the appearance of localized areas of cartilaginous edema (A,B), with complete disappearance of any cellular elements and often with exposure of the fibrillar trama (C,D).

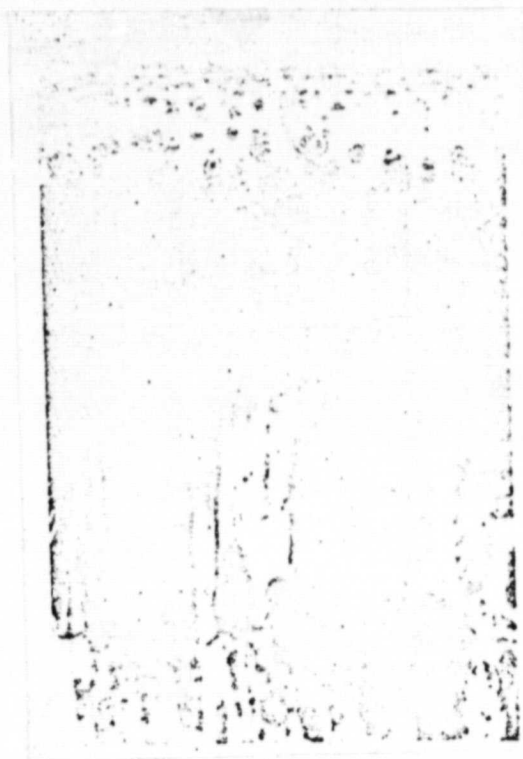


Fig. 13. Group II (immobilization for 40 days). Localized zones of edema are also present in the deepest layers.

Fig. 12. Group II (immobilization for 35 days). Detail of a deep area of asbestiform degeneration.

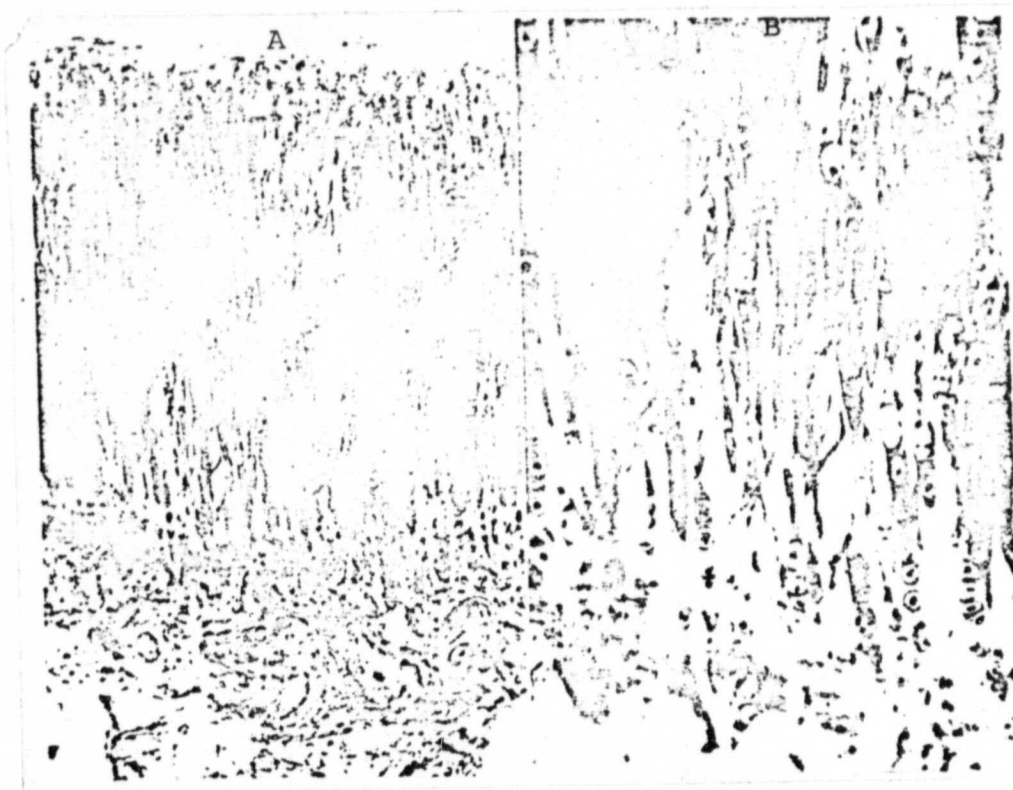


Fig. 14. Group II (immobilization for 40 days). A,B: In some layers of cartilage, the edema appears diffusely throughout the basic substance.

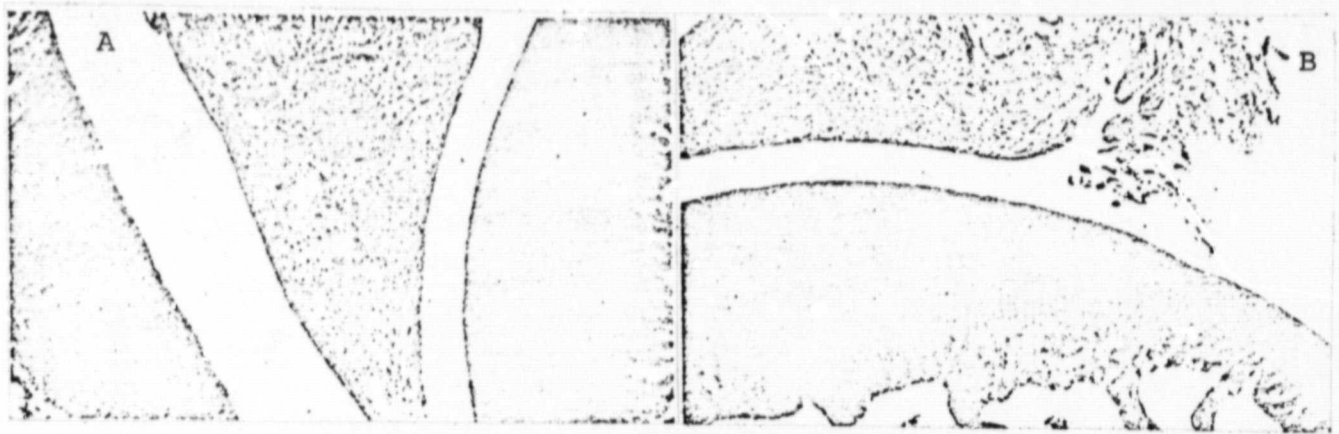


Fig. 15. Group II (immobilization for 40 days). A,B: The fibrous, hypertrophied synovial membrane located between the two articular surfaces.

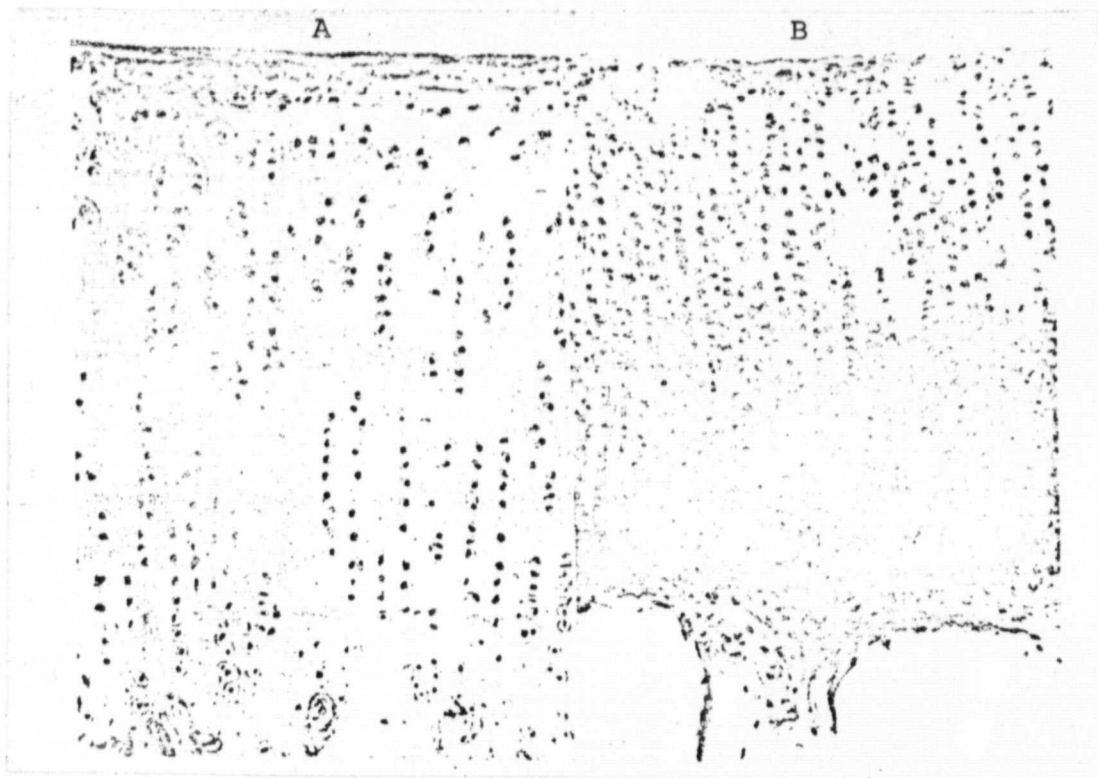


Fig. 16. Group II (immobilization for 50 days). In areas unaffected by degenerative phenomena, the reduction in thickness of the articular cartilage (B) occurs at the expense of the basic substance. The integral cellular elements retain the layered arrangement of the control (A) and, paradoxically, appear to be more numerous.

The cartilaginous cells are still arranged in four characteristic layers. The cells show various signs of involution and disappear in localized areas of destruction of the basic substance. /29

If atrophy of the basic substance is not accompanied by simultaneous reduction in the number of chondrocytes, then the result may be the appearance of an increase in the cellular population (Figure 16).

The subchondral bone is diffusely porous.

Group III (immobilization from 60 to 90 days):

The synovial fluid is even less evident, and the synovial membrane is more fibrous than it was in the animals in Group II.

The articular surface is thinner all over, to the point of almost disappearing in some localized areas (the postero-internal portion of the medial condyle, the posterior portion of the tibial plate and the articular surface of the kneecap) where contact between the two articular heads is maintained (Figure 18, Figure 19).

The reduction in thickness of the articular cartilage occurs at the expense of the superficial layer and of the intermediate layer, which can be distinguished with difficulty. However, the deep layer is well represented, comprising hypertrophic chondrocytes gathered in groups of a few elements (Figure 20, Figure 21).

In other areas, the cartilaginous covering is no longer continuous, and shows numerous longitudinal fissures which extend almost to the layer of calcified cartilage. In the islands of cartilaginous tissue within these fissures, the cells are numerous and regrouped in "colonies" (Figure 22).

In the polar contact zones, the cartilaginous covering can no longer be identified. The subchondral bone is noticeably thicker and sclerotic, and is almost completely exposed (Figure 20). /31

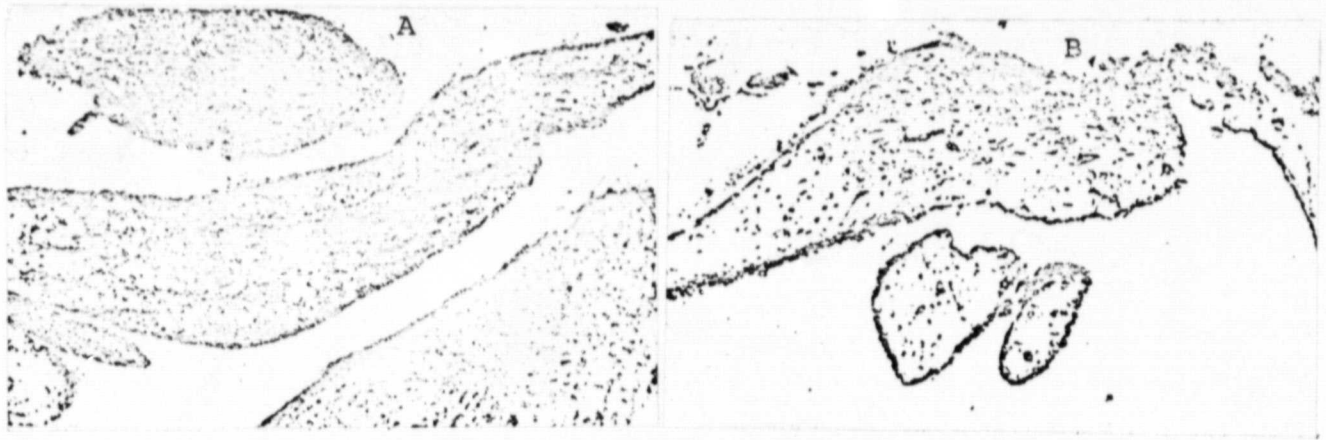


Fig. 17. Group II (immobilization for 58 days). The stromal portion of the synovium of the immobilized joint (A) is more fibrous and vascularized than is the control (B).



Fig. 18. Group III (immobilization for 90 days). There are numerous irregularities in the surface (*) of the treated joint (A). The presence of a depression on the medial condyle (B) (indicated by arrows) is stable.

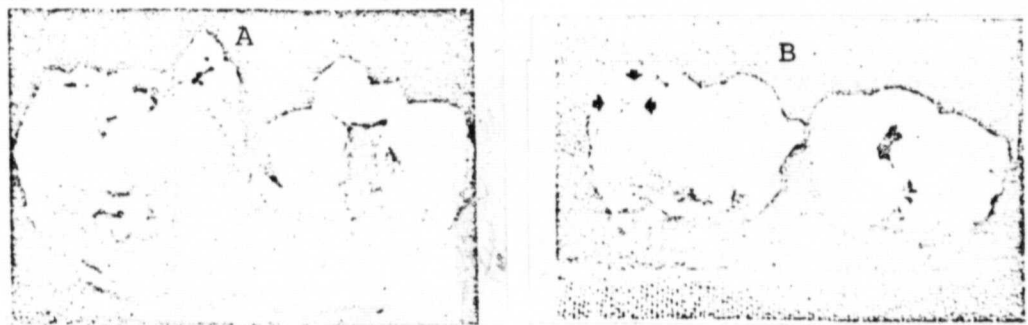


Fig. 19. Group III (immobilization for 90 days). The surface of the tibial plate appears granular and thinned in the immobilized joint (B) as compared to the control (A).

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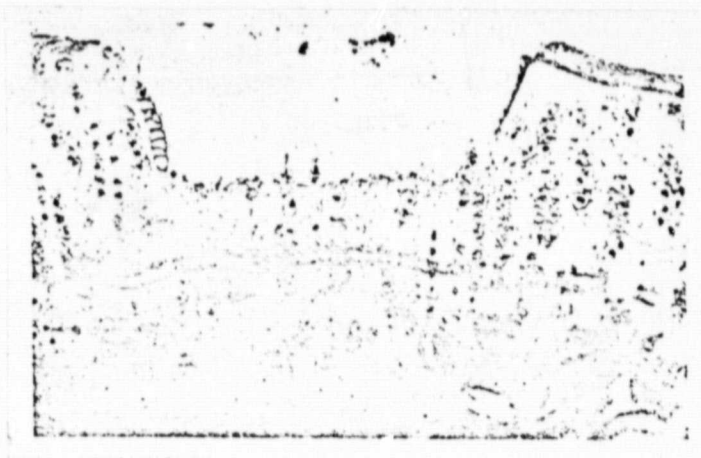


Fig. 20. Group III (immobilization for 90 days). Cartilaginous ulcer affecting the superficial layers.



Fig. 21. Group III (immobilization for 90 days). Atrophied cartilage covered by a fibrous synovial membrane.



Fig. 22. Group III (immobilization for 90 days). The reduction in thickness of the articular cartilage occurs at the expense of the superficial layer (A). Attritive surfaces with extensive fissuring which deepens toward the subchondral bone (B). The chondrocytes appear numerous and hypertrophied at the edges of the fissured parts (C). The irregularity of the surface is often accompanied by serious degenerative phenomena of the basal layers (D).

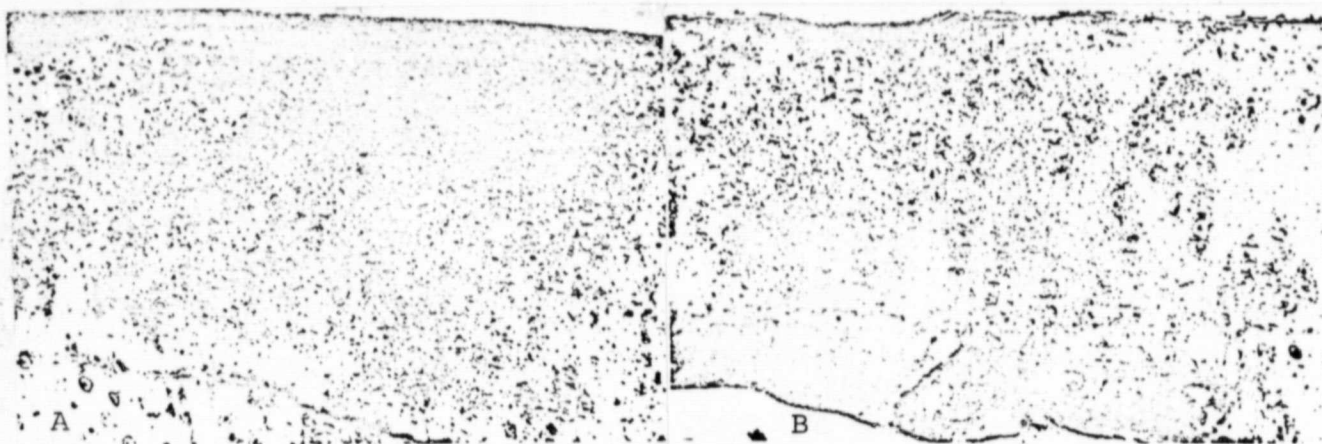


Fig. 23. Group III (immobilization for 90 days). Autohistoradiography: S^{35} uptake is intense in control articular cartilage (A) and to a great extent affects the deep and intermediate layers, which represent the most metabolically active portions. S^{35} uptake appears to be lesser in treated articular cartilage (B).

Group IV (immobilization from 90 to 120 days and longer):

Immobilization for a period longer than 90 days does not cause further changes in the morphology of the articular cartilage or of the other constituent elements of the joint. In fact, the lesions which occur repeat the characteristics of the lesions observed in Group III: diffuse thinning of the articular cartilage and localized areas in which the cartilage itself disappears. /32

The cartilaginous context often reveals areas of necrosis, with complete disappearance of the cellular elements and persistence of a diffusely eosinophilic substance (Figures 27, 28 and 29).

Autohistoradiography

The uptake of S^{35} by the articular cartilage is compromised by immobilization.

As mentioned above, uptake of this isotope is a principal characteristic of the cartilage wherever there is intense biosynthesis of chemical compounds containing sulfur (glycosaminoglycanes).

Studies by Böstrom and Mansson (1953), Boyd and Neuman (1954) and Curran and Gibson (1956) have led to the conclusion that sulfur uptake by the cartilage is a vital function of the chondrocytes and constitutes a measurement of the significance of this process. Supporting this hypothesis is the in vitro research carried out on cartilaginous fragments incubated in a medium containing significant quantities of S^{35} , by McElligott and Collins (1960) and Meachim (1964).

Fixation of radioactive sulfur increases in intensity proportionally to the rate of activity in processes for synthesis of chondroitin sulfuric acid. In fact, radioactivity is at maximum intensity in sections of embryonic cartilage, and is directly proportional to the cellular multiplication period and to the period of formation of a growing quantity of basophilic matrix.

As demonstrated by autohistoradiography, S^{35} uptake by normal articular cartilage characteristically follows a topographic distribution pattern. The intermediate and deep layers, which are the most basophilic and metachromatic, capture the isotope with greater intensity (Figure 23). In these layers, as in the others, the territorial area is the most captive part (Figure 24).

Immobilization causes a reduction of S^{35} uptake in all layers of the articular cartilage. This phenomenon is however most evident in the intermediate and deep layers (Figure 23 and Figure 24). /34

Among animals in Group IV, no subsequent reduction in isotope uptake was noted beyond the stabilized values observed in animals in Group III.

In the areas of localized edema, the incorporation of the isotope was completely absent (Figure 25).

The presence of a hypercaptive zone was noted at the edges of such areas.



Fig. 24. Group III (immobilization for 90 days). Autohistoradiographic behavior can be superimposed on that of basophilia and metachromasia. The territorial area (A,C) captures the isotope with greater intensity than does the interterritorial area. In immobilized cartilage (B,D), the territorial area is less captive than is the control.

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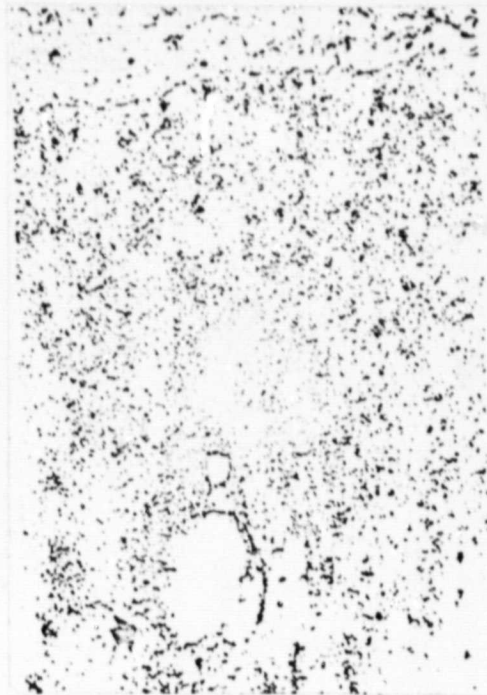


Fig. 25. Area of localized edema: incorporation of the isotope within a lacunar area. Hypercaptive cellular elements are visible at the edges.

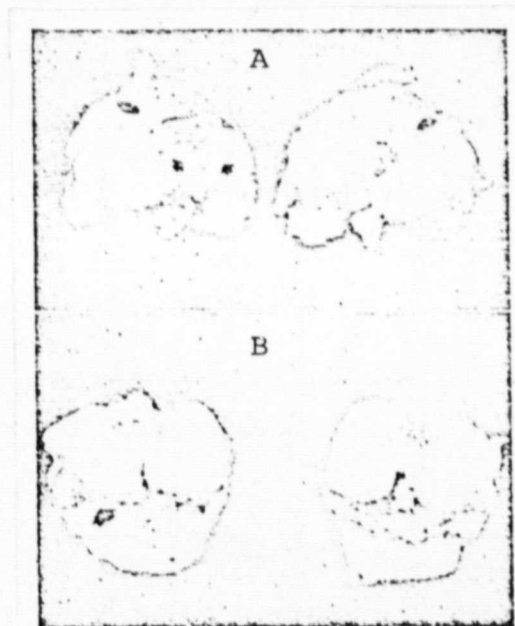


Fig. 26. Group IV (immobilization for 114 days). The articular surface of the internal tibial hemiplate is diffusely thinned (A), but does not disappear. The internal condyle shows marginal surface erosion (indicated by arrows).



Fig. 27. Group IV (immobilization for 114 to 132 days and longer). Affection of the cellular complex is manifested by localized areas (A) or diffuse areas (B,C,D) of necrosis, which become especially pronounced in relation to attritive surfaces.

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Fig. 28. Group IV (immobilization for 132 days). Zones of necrobiosis reappear with fibrous cartilage.

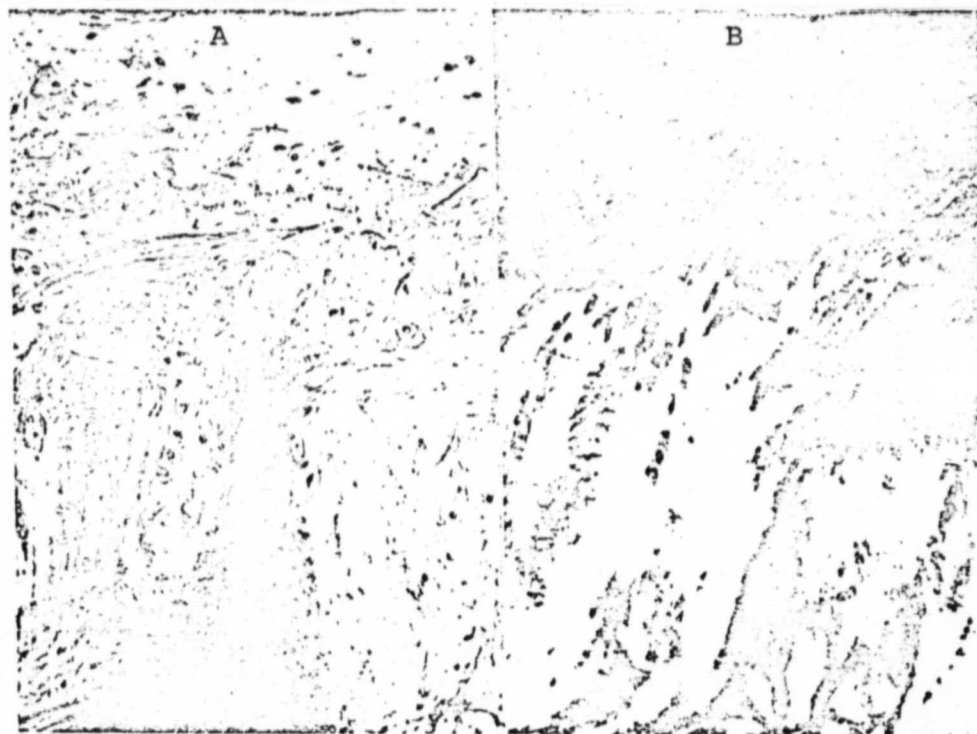


Fig. 29. Group IV (immobilization for 132 days). A: Detail of Fig. 28. B: Polarization reveals the preponderance of the fibrillar component over the basic substance. Transverse bands of collagen fiber are visible.

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Discussion

Prevention of articular excursion, as achieved by immobilization in a plaster device, causes significant changes within the joint. The epiphyseal cartilage, due to the peculiarity of its nutritional mechanism, is one of the first structures affected by lack of movement. This lack of movement also affects other articular components, such as the synovial membrane and the subchondral bone, which are closely connected to and depend upon the overlying cartilage.

Immobilization for less than ten days does not appreciably affect the morphology of the articular cartilage [emphasis in Italian text].

Not even an electron microscope (Roy, 1970) succeeded, however briefly, in revealing significant alterations of the cellular bodies, the basic substance or the collagen structure.

The first signs of deterioration of the cartilaginous tissue begin to appear only after ten days, in the form of variations in the dye receptivity characteristics of the intercellular matrix. The basophilic behavior of the basic substance is less evident in proportion to diffuse or localized areas in which a tint inversion can be observed. /35

The reduced affinity for basic dyes results from modifications of the macromolecular aggregates which constitute the basic substance. Immobilization causes a substantial decrease or at least a variation in the physico-chemical characteristics of the chondromucoids and the keratomucoids, which represent the essential chemical components of the intercellular substance.

In these initial phases, the prevention of movement affects functional activity more than it does the morphology of the cartilaginous cells. /36

Use of autohistoradiography confirms this hypothesis. The uptake of S^{35} by the cartilaginous tissue of the immobilized joint decreases with respect to the control.

Taking into consideration the close relationship between S^{35} deposition and glycosaminoglycane biosynthesis (chondrosulfuric acid; keratosulfuric acid), it must be inferred that the production of this substance by the cartilaginous cells decreases following immobilization.

Protracted immobilization causes more pronounced changes in the basic substance, which appears to be noticeably reduced with respect to the cellular component. However, the articular cartilage undergoes general atrophy, with a relative increase in the cellular population, which has the effect of preserving the apparent architecture of normal cartilage.

Changes in the basic substance ultimately result in localized areas of edema with exposure of collagen fibers, or of asbestiform degeneration.

Cartilaginous cells are also ultimately damaged by the absence of movement. Cellular involution and degeneration appear more frequently after the sixtieth day of immobilization (animals in Group III and Group IV).

In the polar contact zone between the articular surfaces, in which load pressure is maintained, necrotic processes occur, with complete disappearance of the cartilaginous cells.

Our experiments confirm the observations of other researchers (Salter and Field, 1960; Trias, 1961; Crelin and Southwick, 1964; and Roy, 1970). Degeneration and cellular necrosis are often encountered during prolonged periods of immobilization, both in areas of polar contact and in unapposed areas. Changes occur early and are serious when apposition between the two articular surfaces is continuous.

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The radioactive behavior of the sections in these advanced stages of immobilization indicates a subsequent reduction of the isotope uptake; however, [it is] completely absent in the zones of edema or necrosis which surround the hypercaptive parts and which correspond to cellular elements with especially high metabolic activity.

Following a given period of immobilization (which has been calculated to be approximately 120 days), the lesions on the articular cartilage stabilize and do not appear to develop further.

Changes in the subchondral bone and in the synovial membrane follow the same pattern as do the changes in the articular cartilage. /38

The subchondral bone [emphasis in Italian text] shows constantly greater porosity. The medullary spaces are wider than those of the control, and (in the non-weight-bearing zones) contain numerous ectasic vascular formations. The latter sometimes project into the basic substance of the basal layer, after having penetrated the calcified cartilage. In the zone located along the load lines, where contact continues between the articular heads, the subchondral bone thickens.

The fibrous transformation of the stromal axis of the synovial membrane [emphasis in Italian text] causes the decrease in secretory activity of the overlying cells.

As emphasized above, immobilization of the articular segments causes a number of related changes in the epiphyseal cartilage, which are based on a compromise of the functional activity of the cartilaginous cells.

The compromise of cellular activity results from disturbance of chondrocyte nutrition processes which, as noted above, pass the substances required for energy production through the synovial fluid. /39

(In fact, nutrition via the synovial fluid is the fundamental nutritional mechanism of the articular cartilage. The synovial fluid

is the intermediary between the vascular area of the membrane and the articular cartilage, i.e., the vehicle which supplies blood to the cartilage, and vice-versa.)

(The nutritive substances are transported through the basic substance to the cellular complex, where they are assimilated and metabolized. An analogous exchange takes place in the opposite direction for cellular catabolism products.)

The permeability of the basic substance to metabolites varies, depending on its chemical composition and on the electrical state of the glycosaminoglycans.

The permeability of the basic substance is also determined by mechanical functions. Movement is one of the principal mechanical factors /40 which can affect the circulation of the fluid within the cartilage, promoting defluxion and discouraging stanching.

Penetration of the fluid is facilitated by intermittent compression of the articular surfaces, so as to provide a pumping mechanism. During compression of the two articular surfaces, the cartilaginous tissue, which is remarkably elastic, is squeezed, and emits a certain amount of interstitial fluid. When the pressure is removed, the cartilage expands to its initial shape.

Thus, the cartilage has an aspirating effect on the synovial fluid. This effect is facilitated by the negative pressure existing in the articular cavity. Thus, the motion represents a physiological massage which ensures that the same portions of the articular surfaces do not remain in constant reciprocal contact. In this way the movement enables realization of the physiological pumping mechanism.

Immobilization causes a change in this complex and delicate mechanism, whereby the nutrition itself of the cartilage is compromised. This /41 effect is compounded by alteration of the synovial fluid due to changes in the extent of the synovial membrane.

The nutritional deficiency first affects the functional activity of the cartilaginous cells.

The loss of condition during protracted immobilization drains the vitality of the cellular elements, which show varying degrees of involution, often leading to the death of the cells themselves.

In conclusion, alteration of the nutritional exchanges between the synovia and cartilage accounts for the networks of lesions observed during articular immobilization.

Furthermore, beyond the 120-day limit, the articular cartilage succeeds in attaining a certain degree of energy production, by means of an adaptation mechanism which is still unknown.

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